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Comparison Between Soft and Conventional Flexible Antagonist Protocols Regarding Endometrial Receptive Parameters for Women With Poor Ovarian Response and Effect on Pregnancy Rate.

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ABSTRACT

Poor ovarian responders (PORs) are characterized by decreased the response of the ovaries to stimulation. Nowadays, using soft protocol of low doses of Gn with/without oral compounds such as letrozole was accepted as a good option. The endometrium receptive parameters include sub endometrial resistance, pulsatility index and endometrial thickness. Eighty infertile POR women divided to two equal groups and undergoing IVF/ICSI antagonist protocols. One group underwent soft protocol using letrozole 2.5mg twice daily for 5 days starting from cycle day 2-3 overlapped low-dose Gn (225 IU) from cycle day 4-5. Other group received high dose Gn only (450 IU) from cycle day 2-3. Both groups continued stimulation cycle until the day of trigger using the flexible antagonist protocol. Estimation of values of sub endometrial RI and PI with endometrial thickness by 2 Color Doppler ultrasound was performed at day of trigger, OCP and ET. There was no significant difference for endometrial thickness at day of trigger, OCP and ET. There was significant difference in means of sub endometrial RI and PI at day of trigger, OCP and ET (P>0.05). This study showed evidence in favor of soft protocol for POR regarding pregnancy rates in spite of the statically difference in means of sub endometrial RI and PI associated with much less cost effectiveness.

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KEYWORD

Poor Ovarian Responders, Soft Stimulation, Conventional Stimulation

1. Introduction

Soft stimulation protocol involved stimulation of the ovaries with less doses of gonadotrophins and/or other oral compounds like letrozole, while conventional protocol relies on the concept that increasing the doses of gonadotrophins, leading to obtain more oocytes and hence, the more oocytes, the better pregnancy rate. (Drakopoulos,et al. 2016 [1]). The live birth of a single and healthy infant is the aim of the recent assisted reproduction technology (ART). This aim is intended to be accomplished with less time and cost to increase patient satisfaction and safety. In spite of the rapid progress that has been made in ARTs over the period of the past 40 years, there are still several issues that have not been resolved like the problem of the clinical management of patients who have a poor ovarian response (POR) which is still a challenge in everyday practice and disappointing for both the patient and the fertility expert (Drakopoulos et al. 2016 [1]) There have been numerous trials done for the management of POR using different protocols, but these efforts have not been successful in identifying the exact protocol which is most effective (Drakopoulos et al. 2016 [1]). The heterogeneity of this group of women may be the reason behind the difficulty in identifying the most effective strategies in the management of POR (Errázuriz J. et al. . 2019 [2]). Human implantation is a complex process requiring synchrony between a healthy embryo and a receptive endometrium. Diagnosis of endometrial receptivity with most available tests have been subjective and not so accurate in predicting the receptivity. Nowadays using, ultrasound as a non-invasive technique with some limited parameters to assess changes in the endometrium during stimulated cycles. Those parameters include endometrial thickness and sub-endometrial blood flow indicies (RI and PI) measurement. The pulsatility index (PI) represents the strength of blood flow through the

uterine arteries and then to sub endometrium and represents the difference between the peak systolic velocity minus end-diastolic velocity divided by the mean velocity recorded throughout the cardiac cycle and following this formula: PI = (PSV - EDV) /MV where MV=PSV+ EDV/2. It can be classified as low (0.00–1.99), medium (2.00–2.99), and high (over 3.0). The RI is calculated as the ratio of peak systolic flow velocity minus end-diastolic velocity divided by peak systolic velocity, ranging from 0.0 to 1.0 and following this formula: RI = (PSV - EDV) / PSV, Where PSV= peak systolic velocity and EDV= end-diastolic velocity. (Martins et al. 2019 [3]). Subendometrial blood flow includes vessels supplying endometrium within 10 mm of the lateral endometrium edge. Subendometrial resistence (RI) and uterine artery resistance (pulsatility index) are good predictors of endometrial receptivity in ART cycles (Ali zarad C et al. 2021 [4]). The endometrial blood supply arises mainly from the myometrium and, to some extent, from collateral circulation between uterine and ovarian vessels. Therefore, the real blood flow to the endometrium is not from the uterine artery blood flow alone, leading to assess the endometrial blood flow directly by 2 Dimentions Doppler (2D) ultrasound in the spiral arteries adjacent to the endometrium or measurement of blood flow in the sub-endometrium area (Zhang C, H et al. 2022 [5]).

2. Patients and Methods

This is a prospective randomized controlled trial conducted in the IVF center of the High Institute for Infertility Diagnosis and Assisted Reproductive Technology / AL-Nahrian University from November 2020 to May 2022. It includes 80 infertile poor responder women according to Bologna criteria undergoing ICSI protocols. They were divided to two equal groups and undergoing IVF/ICSI antagonist protocols. One group underwent soft

protocol by using letrozole 2.5mg twice daily for five days starting from cycle days 2-3, which overlapped low-dose Gn (225 IU) from cycle days 4-5. Other groups received high dose Gn only (450 IU) from cycle days 2-3. Both groups continued the ovarian stimulation cycle until the day of trigger using the flexible GnRH-antagonist protocol in which all women were received 0.25 mg/day Cetrorelix when at least two or more follicles reached a size of 12-14 mm. Basal physical examination and basal hormonal assessment was performed, including FSH, LH, and S. prolactin, TSH, E2, P4, and AMH associated with AFC by transvaginal ultrasound. At the day of trigger, day of oocyte pickup, and day of embryo transfer, endometrial thickness was measured, and then sub-endometrial blood flow indices, including RI and PI, were also recorded by two dimensions' color Doppler. The data was analyzed using Statistical Package for Social Sciences (SPSS) version 25 using Pearson Chi-square test ($\alpha 2$-test). Independent t-test and Analysis of Variance (ANOVA) (two-tailed) was used to compare the continuous variable accordingly.

3. Results

1- **Demographic Characteristics of Studied Groups**

The mean age of the soft protocol group was 37.05±5.64 years, and 19 patients (47.5%) aged ≥ 40 years. In the conventional protocol group, the mean age was 38.20±4.89 years, and 23 patients (57.5%) aged ≥ 40 years. Regarding infertility, the highest proportion of the studied patients had primary infertility: 26 patients (65%) in the soft protocol group and 25 patients (62.5%) in the conventional protocol group. The most common duration of infertility was 1–4 years in 16 patients (40%) of the soft protocol group and 22 (55%) of the conventional protocol group. Both groups had comparable baseline characteristics, and there were no significant differences (P>0.05) between the two studied groups in regard to age, type of infertility, and

duration of infertility. Also, the mean of BMI of the soft protocol group was 26.34±3.31 Kg/$m^{2,}$ while for the conventional protocol group, it was 25.05±3.55 Kg/$m^{2,}$ and the P value was 0.096 (no significant difference, P>0.05) as shown in (Table 1).

2- Comparison of Subendometrial Blood Flow Analysis and Endometrial Thickness on Day of Trigger

Concerning the assessment of endometrial blood flow, patients in the soft protocol group had a significantly lower mean RI than the conventional protocol group (0.59±0.15 vs. 0.65± 0.07, P=0.024) and significantly lower mean PI than the conventional protocol group (0.95± 0.15 vs. 1.06, P=0.031), while no significant difference in endometrial thickness (P= 0.724) between the two groups (Table 2).

3- Comparison of Receptive Parameters at the Day of Oocyte Pickup

At day of oocytes pickup, patients in the soft protocol group had a significantly lower mean RI compared with conventional protocol group (0.54 ± 0.10 vs.0.85± 0.07, P=0.026), significantly lower mean PI than conventional protocol group (0.77± 0.12 vs. 0.82± 0.09, P= 0.035), the mean endometrial thickness was not significantly different (P=0.988) between the studied groups (Table 3).

4- Comparison of Receptive Parameters at the Day of Embryo Transfer

Comparison between the two study groups by sub-endometrial blood flow analysis on the day of embryo transfer showed significantly lower mean RI in the soft protocol group than the conventional protocol group (0.57± 0.12 vs. 0.62± 0.07, P=0.024) and lower mean PI than conventional group (0.81± 0.13 vs. 0.90± 0.23 P= 0.034), while no significant difference in endometrial thickness between the study groups (P=0.462) (Table 4).

Table 1: Demographic characteristics of the study groups

Demographic characteristics	Study Groups		P- Value
	Soft Protocol Group no. (%) n=40	**Conventional Protocol Group no. (%) n=40**	
Age (Years)			
Mean ± SD	37.05 ± 5.64	38.20 ± 4.89	
< 30	3 (7.5)	3 (7.5)	**0.736**
30 – 34	7 (17.5)	7 (17.5)	
35 – 39	11 (27.5)	7 (17.5)	
≥ 40	19 (47.5)	23 (57.5)	
Type of Infertility			
Primary	26 (65.0)	25 (62.5)	**0.816**
Secondary	14 (35.0)	15 (37.5)	
Duration of Infertility (Years)			
1 – 4	16 (40.0)	22 (55.0)	**0.275**
5 – 9	14 (35.0)	8 (20.0)	
≥ 10	10 (25.0)	10 (25.0)	
	Mean ± SD	**Mean ± SD**	
BMI (kg/m^2)	26.34 ± 3.31	25.05 ± 3.55	**0.096**

no.: number, SD: standard deviation, BMI: body mass index

Table 2: Comparison of Receptive Parameters at the Day of Trigger

Receptive Parameters at the Day of Tigger	Study Groups		P – Value
	Soft Protocol Group no. (%) n=40	Conventional Protocol Group no. (%) n=40	
Endometrial Thickness (mm)	8.92 ± 1.19	8.83 ± 1.20	0.724
RI	0.59 ± 0.15	65 ± 0.07	0.024
PI	0.95 ± 0.15	1.06 ± 0.28	0.031

RI: resistance index, PI: pulsitality index, mm: millimeter

Table 3: Comparison of sub-endometrial blood flow analysis and endometrial thickness at the day of oocyte pickup (OCP)

Receptive parameters at the day of OCP	**Study Groups**		**P – Value**
	Soft Protocol Group no. (%) n=40	Conventional Protocol Group no. (%) n=40	
Endometrial Thickness (mm)	9.31 ± 1.51	9.31 ± 1.38	0.988
RI	0.54 ± 0.10	0.65 ± 0.07	0.026
PI	0.77 ± 0.12	0.82± 0.09	0.035

Table 4: Comparison of Receptive Parameters at the Day of Embryo Transfer

Receptive parameters at the day of ET	Study Groups		P – Value
	Soft Protocol Group no. (%) n=40	Conventional Protocol Group no. (%) n=40	
Endometrial Thickness (mm)	9.41 ± 1.47	9.65 ± 1.43	0.462
RI	0.57 ± 0.12	0.62 ± 0.07	0.026
PI	0.81 ± 0.13	0.90 ± 0.23	0.034

RI: Resistance index, PI: pulsitality index

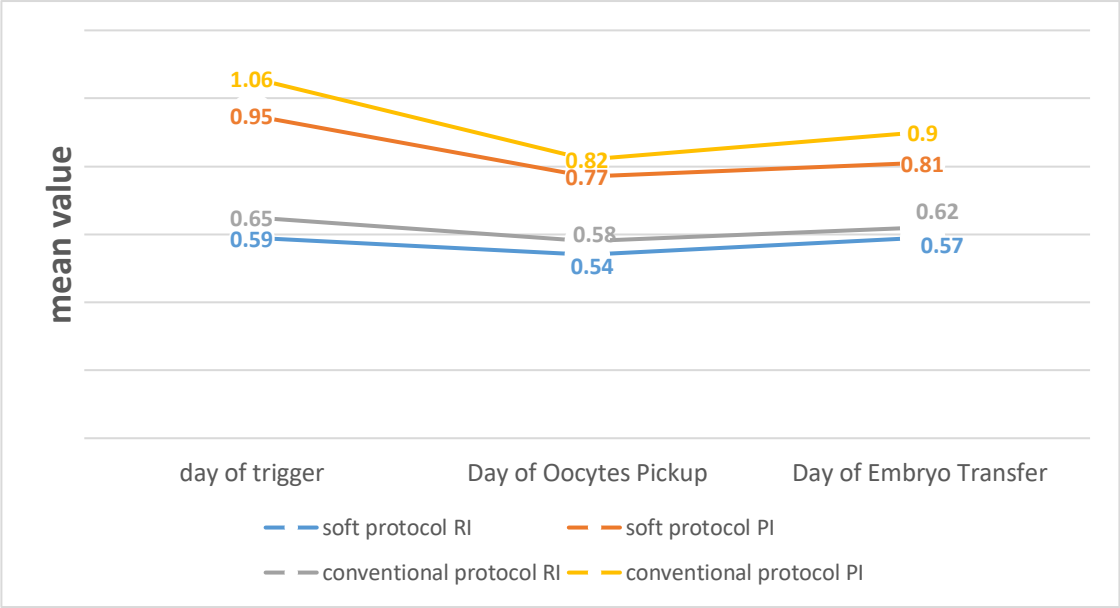

Figure-1: Comparision of subendomertrial RI: Resistance index, PI: pulsitality index

Table 5: Comparison Between Study Groups According to Pregnancy Outcome

Clinical Pregnancy	Study Groups		P- Value
	Soft Protocol Group no. (%) n=40	Conventional Protocol Group no. (%) n=40	
Positive	4 (10.0)	7 (17.5)	0.560
Negative	23 (57.5)	27 (67.5)	

4. Discussion

Endometrial receptivity in the ART cycle presents a challenge for fertility experts and needs correct data to get better treatment strategies. Ultrasound developments helped to give more information about the morphokynetics of the endometrium and its changes throughout the cycle that enables it to become receptive to a healthy embryo.

1. Demographic Characteristics of Study Groups

Regarding the demographic features of women involved in this study, which include age, duration of infertility, types of infertility, and BMI, there was no significant difference between them (Table 1). This means that our result will depend on our interference since all women involved in this study are poor responders selected according to bologna criteria to decrease the rate of bias. Age is one of the most significant factors that predict the success of IVF cycles because it is associated with a reduced number and quality of oocytes (McLernon et al. . 2016 [6]).

2. Comparison of Ultrasound Receptive Parameters at Day of Trigger of the Study Groups

In the current study, the mean RI and PI of the soft protocol group was considerably lower than the conventional protocol group and showed a significant difference between the study groups at the day of the trigger. The highest means of RI and PI for both groups were presented at the day of the trigger. This result matched a study for Silva and et al. ., which was a prospective case-control study. They measured RI and PI serially on days 2, 3, 6, 8, 10, the trigger day, and embryo transfer day in a group of 169 women who were being monitored using an antagonist protocol. They noticed that during the ovarian stimulation cycle, sub-endometrial blood flow showed a similar pattern with comparable values at baseline and increasingly higher values during stimulation, reaching the highest level on the day of trigger and then a significant decrease after hCG administration and recovery to nearly the previous values at embryo. These findings may have some connection to the hormonal state that was present during controlled ovarian stimulation and the impact of elevated serum estradiol levels (Martins et al. 2019 [3]). This result agreed with the result of the current study, which showed the same track in RI and PI measurement. Silva Martins explained the maximum increment in RI and PI associated with the increment of estradiol level reaching its peak level at trigger day and declined after hCG triggering until the day of embryo transfer. (Martins et al. 2019 [3]). Estrogen can affect the vascular smooth muscle and endothelial cells and has a direct effect on the vessel wall (Lee CH et al. 2017 [7]). (Yu Ng EH, et al. 2006 [8]) Said that no information is present in the literature concerning the factors affecting the blood flow toward the endometrial and sub-endometrial regions during IVF. Therefore, they perform a study to evaluate the effect of women's age, their type of infertility (i.e., primary or secondary) and parity, causes of infertility, and serum

estradiol (E2) levels on the endometrial and sub-endometrial regions. They found that only the serum E2 concentration was negatively affect endometrial and sub-endometrial blood flow during IVF treatment in spite of the potent angiogenic and vasodilatation effects of E2. The exact mechanisms by which estradiol exerts its effect on both physiological and pathological angiogenesis is still uncertain, and supraphysiological E2 concentration may have opposite effects on endometrial angiogenesis. In IVF/ICSI, cycle endometrial and subendometrial blood flows were significantly lower than that in the natural cycle. Estradiol had a tow wave effect on the expression of vascular endothelial growth factor mRNA in endometrial cultures, which was up-regulated by E2 at the concentration of 36.7-183.5 pg/ml but downregulated by E2 at the concentration of 360.7 pg/ml (Yu Ng EH et al. 2006 [8]) This explains our result regarding significant reduction in RI and PI in soft protocol group.

3. Comparison of Ultrasound Receptive Parameters at Day of Oocytes Pickup of Study Groups

At the day of OCP, there was no significant difference in concerning endometrial thickness (P= 0.988) because no more Gn was administered after triggering with HCG, but still, there was the difference in RI with mean ± SD 0.57 ± 0.12 for soft protocol versus 0.62 ± 0.07 for conventional protocol and P= 0.026 while PI had no significant difference p=0.278. (Martins et al. 2019 [3]). Mentioned that a significant decrease of RI and PI values after hCG administration at the day of oocyte pickup but go back to what was seen throughout the stimulation cycle before the trigger day, reaching the lowest values at the day of oocyte pickup due to the effect of hCG in increasing the vascular endothelial growth factor (VEGE) expression.

4. Comparison of U/S Receptive Parameters at the Day of Embryo Transfer of Study Groups

At the day of embryo transfer, showed no significant difference in the mean endometrial thickness, mean RI, and mean PI between the two groups (P>0.05). In the contrary, the results observed in Sekhon et al. study reported that the letrozole group had thicker endometrium at the time of embryo transfer (p<0.05) (Hu YJ et al. 2024 [9])

Sub-endometrial blood flow analysis (resistance and pulsatility index) showed a statistical difference between the two groups at the day of ET in the study groups. Silva Martins observed that there was a statistically significant difference between the participants who utilized hCG throughout that time period, both at the trigger and on the day of embryo transfer (P 0.001). It is possible that the influence that hCG has on vascularization is responsible for the decrease in both RI and PI due to the enhancement of angiogenesis. This is because hCG has an up-regulating effect on the vascular endothelial growth factors. Therefore, RI and PI start to increase again at the day of embryo transfer but do not reach to the level of the day of trigger. Measurements of sub-endometrial blood flow, either individually or in combination with uterine artery flow indices, may help in the assessment of the receptivity of the endometrium to the embryo to enhance the selective single embryo transfer (Martins et al. 2019 [3]).

5. Comparison of Pregnancy Outcome

There was no significant difference in pregnancy rate in spite there was significant differences in RI and PI means in favor of soft protocol between the study groups. PR in the soft protocol group (10.0%) was lower than in the high dose group (17.5%), but the difference in the pregnancy rate was not significant. Tal Lazer disagrees with our findings and came to the conclusion that using a soft protocol was more effective than using a conventional

protocol of stimulation in improving clinical pregnancy and live birth rates because too much Gn has a negative effect on the endometrium rather than on oocyte quality as well as the significant modulation of genes and altered gene expression profiles that participate in processes of implantation (Tal Lazer et al. 2014 [10]). Another study published by Bülow 2021 study who reported that ongoing pregnancy rate was similar between both groups (31% vs 39% (p = 0.55) (Bülow NS et al. . 2022 [11]).

6- Conclusion

The current study showed evidence in favor of soft protocol for POR since it is resulted in no difference in pregnancy rates in spite of the statically difference in means of sub-endometrial RI and PI associated with much less cost-effectiveness.

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Author Contribution

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Conflict of Interest

The author declares no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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